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STUDIES TO CONTROL ENDEMIC TYPHOID FEVER IN CHILE

ANNUAL REPORT

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SUMMARY

A multi-faceted program of applied research has been undertaken in collaboration with the Ministry of Health of Chile intended to lead to control of endemic typhoid fever in Santiago, Chile. Information derived from these studies is directly applicable to the prevention of typhoid fever in United States military personnel deployed in endemic areas.

During the past contract year, activities that were emphasized include:

- 1) Maintenance of prospective epidemiologic and bacteriologic surveillance in three large-scale field trials evaluating the efficacy of Ty21a live oral typhoid vaccine given in various formulations immunization schedules.
- 2) Epidemiologic studies to ascertain the modes of transmission of typhoid fever in Santiago, Chile to identify intervention points.
- 3) Evaluation of a highly specific serological assay that measures Vi antibody as a screening test to detect the presence of chronic Salmonella typhi carriers in epidemiologically important populations such as foodhandlers.

FIELD TRIALS WITH Ty21a

Results of the large-scale field trials of Ty21a involving 456,000 Chilean schoolchildren show that an enteric-coated capsule formulation is significantly superior in efficacy to a gelatin capsule/ NaHCO_3 formulation (Area Occidente trial). One dose of vaccine in enteric-coated capsules provides only low levels of short-lived protection while the moderate (65%) protection conferred by two doses lasts for only two years and drops to insignificant levels thereafter (Area Norte trial). Three

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doses of Ty21a in enteric-coated capsules given within one week provides moderate protection (65%) for at least two years (Area Occidente trial). There was no increased efficacy when the three doses of either enteric-coated capsules or gelatin capsule/ NaHCO_3 formulations of Ty21a were given with intervals of 21 days between the doses. Four doses of Ty21a vaccine in enteric-coated capsules provide significantly superior protection than three doses (Area Sur and Central trial). The live oral typhoid vaccine Ty21a did not cause adverse reactions.

EPIDEMIOLOGIC STUDIES OF THE ENDEMICITY OF TYPHOID FEVER IN SANTIAGO AND
A SEROLOGIC SCREENING TEST FOR IDENTIFYING CHRONIC CARRIERS

Epidemiologic studies were undertaken in Santiago, Chile, where typhoid fever is endemic in school age children, to determine the prevalence of typhoid carriers in foodhandlers in schools and to assess their possible role in transmission of Salmonella typhi. Bacteriologic and serologic (Vi antibodies) tests identified one chronic S. typhi carrier among 177 foodhandlers examined. Although a survey among the foodhandlers showed poor knowledge of the cause and modes of transmission of typhoid, and fingernail cultures of 77% yielded coliform bacteria (suggesting poor personal hygiene), there was no evidence that the annual incidence of typhoid was higher in the school with the carrier (2.9 cases/ 10^3 children) than in schools which had no carriers among their foodhandlers (2.6 cases/ 10^3 children). These data suggest that school food handlers are not important in maintaining the endemicity of typhoid in Santiago, which agrees with the observation that most transmission occurs in summer when schools are not in session.

FOREWORD

For the protection of human subjects the investigator(s) have adhered to policies of applicable Federal Law 45CFR46.

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I. INTRODUCTION

Typhoid fever which remains endemic in many less-developed regions of the world poses a potential health risk for travelers from industrialized, sanitized environments who visit such endemic areas. Consequently, for United States military personnel who are stationed in less-developed areas or must be prepared at short notice to operate in less-developed areas of geopolitical importance, typhoid fever represents an important potential health risk. The current vaccine utilized by the U.S. military forces to prevent typhoid fever, an acetone-inactivated preparation of whole Salmonella typhi inoculated parenterally, requires at least two doses given several weeks apart to immunize and causes high rates of significant adverse reactions. Therefore, a high priority has been given to identifying alternative typhoid vaccines that will provide significant protection without causing notable adverse reactions.

In areas where typhoid fever is endemic, the prevalence of chronic gall bladder carriers of S. typhi is often quite high. Thus, a particularly onerous risk of transmission of typhoid fever to U.S. military personnel in less-developed areas comes from foodhandlers in the indigenous population who may be chronic typhoid carriers and who unknowingly are involved in preparation of food. Under these circumstances, unwittingly, the potential exists for large epidemics to occur. Furthermore, the size of the inocula of S. typhi present in food vehicles may be sufficiently high to overcome the protective efficacy of the current acetone-inactivated parenteral vaccine. Consequently, a simple, practical yet sensitive and specific screening test is required to screen large groups of individuals for the presence of suspected chronic typhoid carriers.

Dependents, including children, who accompany U.S. military personnel stationed on tours of duty in less-developed countries must also be protected against typhoid fever. In young children the subject of adverse reactions to the current parenteral typhoid vaccines is even more pertinent.

For the past several years, the Center for Vaccine Development of the University of Maryland School of Medicine has conducted an applied research program on the control of typhoid fever in Santiago, Chile, a highly endemic area. During the past two years, the program has, in particular, concentrated on field studies with Ty21a live oral typhoid vaccine, the development of improved serologic screening tests for the chronic typhoid carrier state, the identification of improved non-surgical methods to treat chronic carriers, and initial evaluations of Ty21a vaccine in infants and young children (representing the first experiences with this vaccine in children less than six years of age). Results of these studies have direct relevance for improved prevention of typhoid fever in U.S. military personnel.

II. FIELD TRIALS OF EFFICACY OF LIVE ORAL TYPHOID VACCINE TY21a

A detailed summary of the results of three separate controlled field trials of the efficacy of Ty21a vaccine in involving more than 450,000 school children in Santiago, Chile is contained in APPENDIX A.

III. EPIDEMIOLOGIC STUDIES OF THE ENDEMICITY OF TYPHOID FEVER IN SANTIAGO AND A SEROLOGIC SCREENING TEST FOR IDENTIFYING CHRONIC CARRIERS

In Santiago, Chile, where typhoid fever is highly endemic, the incidence of the disease is highest in school age children. However, the

disease is largely confined to the warm months of the year with peak incidences occurring in summer when children are on school holiday. Nevertheless, a case/control epidemiologic study suggested that when school is in session, some transmission of typhoid fever occurs within schools. Accordingly, an epidemiologic study was carried to assess the food hygiene practices in a sample of Santiago schools as well as to quantitate the level of knowledge about typhoid and its transmission held by a sample of foodhandlers in schools. The foodhandlers were also examined bacteriologically (by two coprocultures) and serologically to detect chronic typhoid carriers. The serological test was a passive hemagglutination assay using highly purified Vi antigen to measure Vi antibody. This represents one of the first systematic applications of this screening test in an endemic area. The results of these studies are summarized in APPENDIX B.

CONTRACT-RELATED PUBLICATIONS

Papers

1. Ferreccio, C., Levine, M.M., Manterola, A., Rodriguez, G., Rivara, I., Prenzel, I., Black, R.E., Mancuso, T., Bulas, D. 1984. Benign bacteria due to Salmonella typhi and paratyphi in children less than two years of age. J. Pediatr. 104:899-901.
2. Sears, S.D., Ferreccio, C. Levine, M.M., Cordano, A.M., Monreal. J., Black, R.E., D'Ottone, K., Rowe, B., Chilean Typhoid Committee. 1984. Isolation of Salmonella typhi from irrigation water in Santiago, Chile using Moore swabs. J. Infect. Dis. 149:640-642.
3. Murray, B.E., Levine, M.M., Cordano, A.M., D'Ottone, K., Jayanetra, P., Kopecko, D., Pan-Urai, R., Prenzel, I. 1985. Possible reasons for this paucity of resistance plasmids in Salmonella typhi. J. Infect. Dis. 151:551-555.
4. Black, R.E., Cisneros, L., Levine, M.M., Banfi, A., Lobos, H., Rodriguez, H. 1985. A case-control study to identify risk factors for endemic typhoid fever in Santiago, Chile. Bull W.H.O. 63:899-904.

APPENDIX A

PROGRESS REPORT

THE EFFICACY OF ATTENUATED *SALMONELLA* TYPHI

ORAL VACCINE STRAIN TY21A EVALUATED IN CONTROLLED FIELD TRIALS

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Typhoid fever remains an important public health problem in many less-developed regions of the world and poses a risk for travelers from industrialized countries who visit such regions. In virtually all endemic areas the incidence rates for typhoid fever are highest in children 5-19 years of age, i.e. schoolchildren. This is of potential importance in terms of disease control, since schoolchildren represent a "captive" population amenable to school-based immunization programs. Until recently, the standard vaccines against typhoid fever have consisted of acetone-killed or heatphenolized whole cell Salmonella typhi bacteria inoculated parenterally. These parenteral killed whole cell vaccines were shown to have variable efficacy in controlled field trials supported by the World Health Organization (1,3,7,10,15,18). Two field trials (Yugoslavia, Poland) (7,18) demonstrated moderate efficacy (79-84% protection) but vaccine efficacy as low as 40% (in a trial on the island of Tonga (15) and as high as 90% (in a field trial in Guyana (1) were also recorded. However, while the parenteral killed whole cell typhoid vaccines have been shown to provide at least moderate efficacy in field trials, they have rarely been utilized as public health tools in control of endemic disease. This is because the killed whole cell parenteral vaccines cause notable systemic (fever, malaise) and local (erythema, induration, pain) adverse reactions in approximately 20% vaccine recipients (1,7,10). Commonly these adverse reactions are of sufficient severity to put the effected individual to bed for a day or two.

An important advance for the potential control of typhoid fever was the development by Germanier and Furer (5) of an attenuated strain of S. typhi, Ty21a, that can be utilized as a live oral vaccine. In preliminary studies in adult volunteers in North America the vaccine strain was shown to cause no adverse reaction (in marked contrast to the parenteral killed whole-cell

vaccines), to be genetically stable, and to significantly protect against experimental infection with an inoculum of pathogenic S. typhi (6) that caused typhoid fever in 53% of control volunteers.

Based on these highly encouraging observations in adult volunteers, the Ty21a vaccine was evaluated for efficacy by Wahdan et al^{9,10} in placebo-controlled, randomized, double-blind field trial in 32,388 6-7 year old school-children in Alexandria, Egypt. Three doses of Ty21a vaccine ($1-3 \times 10^9$ viable organisms per dose) or placebo were given to the children on Monday, Wednesday and Friday of one week. Prior to ingestion of vaccine or placebo, children chewed a tablet containing 1.0 gm of NaHCO_3 (to neutralize gastric acid). Each dose of lyophilized vaccine was contained within glass vials in vacuo. The vials were opened, the vaccine reconstituted in the field with diluent and the vaccine suspension given to the child a few minutes after the child ingested the NaHCO_3 tablet. In this field trial the vaccine was as safe as in North American adults; no adverse reactions were attributed to the vaccine.

During the 36 month period of surveillance in Alexandria vaccine efficacy was 96% (Table 1) (16). The annual incidence rate in the placebo control group was 44-50 confirmed cases per 10^5 schoolchildren (Table 1), and incidence rate markedly lower than that found in many other areas where typhoid fever is endemic.

Shortly after the Egyptian field trial established the biological activity and safety of Ty21a vaccine in schoolage children in an endemic areas, the Swiss Serum and Vaccine Institute made commercially available a formulation of vaccine consisting of two gelatin capsules each containing 0.4 gm of NaHCO_3 and a third gelatin capsule containing lyophilized Ty21a vaccine. Although this formulation resembled that used in the Egyptian field trial, it

was clearly not identical. Despite the highly encouraging results in the first field trial, it was obvious that additional information had to be obtained before the Ty21a live oral vaccine could be employed as a public health tool. Critical further questions to be answered included:

- 1.) Was it possible to successfully deliver Ty21a vaccine in a formulation, such as enteric-coated capsules, that would not require pretreatment with NaHCO_3 , thereby enhancing the practicality for mass vaccinations?
- 2.) Could fewer (one or two) doses of vaccine provide a high level of protection?
- 3.) What level of protection would Ty21a vaccine provide in endemic areas with incidence rates of typhoid fever several fold higher than Alexandria, Egypt?
- 4.) What is the efficacy of the formulation consisting of gelatin capsules containing NaHCO_3 and vaccine that was marketed after the Egyptian field trial?
- 5.) Could prolongation of the interval between doses enhance the immunogenicity of the vaccine?

In order to answer these questions, three separate field trials of efficacy were carried out in Santiago, Chile. These trials represent collaborative effort undertaken by the Ministry of Health, Santiago, Chile, the Center for Vaccine Development of the University of Maryland, the Pan American Health Organization, the World Health Organization, the Swiss Serum and Vaccine Institute and the Walter Reed Army Institute of Research.

MATERIALS AND METHODS

Pilot Projects

Two pilots in Chile involving randomized administration of vaccine or placebo to several hundred adults or children under double blind conditions

established the safety and immunogenicity of vaccine in this formulation. The results are summarized in Table 2. There was no significant difference in adverse reactions in vaccinees versus placebo controls. Furthermore, the seroconversion rate of IgG class O antibody in children immunized with three doses of enteric-coated vaccine was the same as in children who received vaccine in milk with NaHCO_3 (Table 2).

Field Trials

The first two field trials were placebo-controlled and were initiated in the Northern (Area Norte) and Western (Area Occidente) administrative areas of Santiago in 1982 and 1983, respectively. The third field trial was begun in the Southern (Area Sur) and Central (Area Central) administrative areas of Santiago in 1984. Santiago, Chile was selected as the site for these field trials because of the combination of high endemicity of typhoid fever (the annual incidence rate from 1977 to 1981 exceeded 150 cases per 10⁵ population), an internationally-reknowned health care infrastructure (the National Health Service), a strong commitment on the part of the Ministry of Health towards innovative methods to control typhoid fever, and a long history of school-based vaccination programs.

Only children of consenting parents entered the studies and were randomized to the various cells of the trials. Remaining children of non-consenting parents were also kept under surveillance and served as unvaccinated contact controls. Randomization occurred by classroom.

Since typhoid fever exhibits a marked seasonality (November to April) in conjunction with summer in Santiago, the vaccinations were limited to the cool months of the year (May to October). Computerized data files were generated from the completed class lists.

In the Chilean field trials only bacteriologically-confirmed cases (i.e. isolation of S. typhi from blood or bone marrow) were utilized in computations of vaccine efficacy. Therefore much attention and resources were directed toward bacteriologic confirmation of suspect cases. Children admitted to hospital with a clinical suspicion of typhoid fever had three 4 ml blood cultures and one bone marrow culture. Children who presented as outpatients to the consultorios (health centers) with suspect typhoid fever had two blood cultures drawn 30 minutes apart. S. typhi and S. paratyphi A and B isolates were sent for phage typing to the Institute of Public Health, Santiago and to Dr. Bernard Rowe at the World Health Organization Collaborating Center for Phage Typing of Salmonella, Division of Enteric Pathogens, Central Public Health Laboratory, London, England.

Area Norte Field Trial

Parents of 91,954 of the 137,697 schoolchildren in Area Norte gave permission for their children to participate in the first field trial. These children were randomized to one of three groups:

- 1.) One group received two doses of Ty21a vaccine in enteric-coated capsules ($1-5 \times 10^9$ viable vaccine organisms per dose) given one week apart.
- 2.) A second group got one dose of vaccine and one dose of identical-appearing placebo one week apart.
- 3.) The third group received two doses of placebo given one week apart.

The identical appearing vaccine and placebo capsules were coded (A and B) and the vaccination and surveillance were carried out in double blind fashion. The enteric-coated capsules were intended to obviate the need for NaHCO_3 pretreatment. Vaccine or placebo were delivered to participating children in late May and early June and surveillance began on July 1, 1982.

Area Occidente Field Trial

A second field trial of Ty21a vaccine was initiated in Area Occidente in 1983. In this trial 141,127 children of consenting parents (representing 96% of all schoolchildren) in Area Occidente were randomized to one of five groups to receive:

Group 1 - Three doses of vaccine in enteric-coated capsules given with an interval of two days between doses.

Group 2 - Three doses of vaccine NaHCO_3 given with an interval of two days between doses. The commercially available gelatin capsule formulation was used which consisted of two gelatin capsules each containing 0.5 gm of NaHCO_3 and one gelatin capsule containing lyophilized vaccine.

Group 3 - Three doses of Ty21a in enteric-coated capsules with an interval of 21 days between the doses.

Group 4 - Three doses of the commercial gelatin capsule formulation with an interval of 21 days between the doses.

Group 5 - Three doses of placebo given with an interval of two days between doses.

Mass administration of vaccine or placebo was carried out between mid July and mid September, 1983 and surveillance began on September 21, 1983.

Area Sur/Area Central Field Trial

A third field trial was undertaken in 1984 in Area Sur and Area Central where 248,544 children were randomized to receive either two, three or four doses of Ty21a vaccine in enteric-coated capsules with all doses being given within a period of eight days. No placebo control group was included in this trial for which surveillance began on November 1, 1984.

Results

Area Norte Field Trial

The main objectives of the Area Norte field trial were to evaluate the efficacy of one or two doses of Ty21a in an enteric-coated formulation in an area where the annual incidence of typhoid fever was expected to be much higher than in Alexandria, Egypt. The number of children who participated in the Area Norte trial (91,954) was three times larger than in the Alexandria, Egypt field trial. Yet the enteric-coated formulation proved to be very practical and highly suited to mass vaccination. As in previous experiences, the vaccine was very well-tolerated.

The results of almost three complete years of surveillance (33 months) are shown in Table 3. It is obvious that the efficacy of the vaccine varied notably but that two doses of vaccine always gave significantly superior protection than one dose of vaccine. In the first two complete years of surveillance the incidence rates in the placebo group, 210.9 and 141.7 cases/ 10^5 , far exceeded the rates in the Alexandria field trial (44-50/ 10^5). The efficacy of two doses of vaccine correlated with age over the 33 months of surveillance, (Table 4) being lowest in the 5-9 year old age group (48%), intermediate in the 10-14 year age group (55%) and highest in the 15-19 year old group (61%).

Vaccine efficacy was calculated for each three month interval within the total 33 months of surveillance. In this analysis (Table 5), it is apparent that vaccine efficacy was moderate or high (57-100%) in every three month period except April to June 1983 and January to March, 1985 when it was 0%. This suggests that unusual epidemiologic events were operative during those two three month periods, such as circulation of a particularly virulent strain of S. typhi or circulation of vehicle(s) of transmission containing high inocula of S. typhi sufficient to overcome the protective effect of vaccine.

Analysis of the phage types of S. typhi causing disease in vaccines during the three month period of April to June 1983 showed that they did not differ from those isolated from placebo controls, nor was a common plasmid detected. These observations argue against the theory of a highly virulent strain in circulation during that period. The hypothesis involving ingestion of inordinately high inocula during the period of lack of vaccine efficacy cannot be proved or disproved.

During the 33 month period of surveillance two doses of Ty21a vaccine also provided 39% protection against enteric fever due to S. paratyphi B (Table 6). This partial cross-protection is believed to be due to the fact that S. typhi and S. paratyphi B share one O antigen (antigen 12) in common (4) and the efficacy of live Salmonella vaccines is related to their O antigen specificity (11,12,13).

Even in the second year of surveillance of the Area Norte trial, vaccine efficacy against typhoid fever was only 72% versus 96% in the Alexandria, Egypt field trial. Was the lower efficacy in the Area Norte trial the consequence of fewer doses? Or a different formulation? Or the many fold higher incidence of typhoid in Area Norte? A second field trial was initiated in Area Occidente in an attempt to answer some of these questions.

Area Occidente Field Trial

Analysis of the results of the first year of the trial in Area Norte prompted initiation of a second placebo-controlled, randomized trial in Santiago. Area Occidente was selected because it is contiguous to Area Norte and the two administrative areas closely resemble one another in demography, socioeconomic level and health care infrastructure. The 141,127 participating children of consenting parents were randomized into five groups. Results of the first 18 months of surveillance are shown in Table 7. In the first 12 months of surveillance the incidence in the placebo group was 126 confirmed

cases per 10^5 schoolchildren, a rate three-fold higher than Alexandria, Egypt and very similar to the incidence rate ($141.7/10^5$) in the placebo group in the Area Norte field trial during the same calendar period.

In the Area Occidente field trial children received three doses of Ty21a vaccine, as in Egypt. Children received vaccine either in enteric-coated formulation or in the gelatin capsule/ NaHCO_3 formulation and the three doses were administered either with an interval of two days or 21 days between doses. This design allowed a comparison of the formulation used in the Area Norte trial (enteric-coated capsules) with a formulation that includes pretreatment with NaHCO_3 and thereby resembles (although is not identified to) the formulation used in Egypt.

The enteric-coated formulation gave 62% efficacy when vaccine was given with an interval of only two days between the doses and an almost identical efficacy (59%) when the interval between doses of enteric-coated capsules was extended to 21 days.

In contrast, the gelatin capsule/ NaHCO_3 formulation gave very poor protection whether given in intervals of two days (18% protection) or 21 days (30%) between the doses. The difference in incidence rates between recipients of enteric-coated vaccine by either immunization schedule and recipients of the gelatin capsule formulation (by either immunization schedule) is highly significant ($p < 0.0004$).

Results of the Area Occidente field trial showed a clear-cut superiority of the enteric-coated formulation over the gelatin capsule/ NaHCO_3 formulation. The trial also demonstrated that increasing the interval between the doses to 21 days conferred no advantage. This trial has provided necessary information regarding how to utilize Ty21a vaccine as a public health measure: three doses of the enteric-coated formulation given within one week provide moderate (60%) efficacy against typhoid fever in an area of high endemicity

without causing adverse reactions. The critical questions of the duration of immunity conferred by Ty21a in enteric-coated capsules will be answered by maintaining surveillance in the Area Occidente and Area Norte field trials for a period of five to seven years.

Area Sur/Area Central Field Trial

In the second year of surveillance in Area Norte two doses of enteric-coated vaccine given one week apart provided 72% protection when the incidence rate in the placebo control group was 141.7 per 10^5 . During the same chronological period in Area Occidente (which closely resembles Area Norte), three doses of enteric-coated vaccine given within one week conferred 74% protection when the incidence rate in the placebo control group was 122 per 10^5 . Over 33 months and 18 months of surveillance, respectively, two doses of enteric-coated vaccine gave 54% protection in Area Norte, while three doses gave 62% in Area Occidente. These results suggest that there may be little difference in the protection conferred by two or three doses of enteric-coated vaccine. However, this conclusion cannot be fairly drawn by comparing results from two separate trials. Only a direct comparison with randomization of groups can provide a proper answer. Accordingly, a third field trial was undertaken in Area Sur and Area Central of Santiago in 1984 where 248,544 children were randomized to receive two, three or four doses of enteric-coated vaccine with all doses being given within a period of eight days. Surveillance began on November 1, 1984. There was not a true randomized placebo control group in study. The unvaccinated group comprises children who were absent at the time of the initial vaccination and who were then held ineligible from further participation. These children are included in this analysis in order to obtain some measure of absolute vaccine efficacy. In the previous two trials there was no significant difference in attack rate between randomized placebo controls and unvaccinated controls.

Preliminary results of nine months of surveillance in this field area are shown in Table 8. Although preliminary, the data already show that four doses of vaccine give significantly greater protection than two doses; furthermore, the trend suggests that four doses are superior to three doses of vaccine. The data also suggest that four doses of vaccine were giving moderate protection even when two or three doses were not.

DISCUSSION

Parenteral killed whole cell typhoid vaccines are efficacious but cause unacceptable adverse reactions (1,3,7,10,15,18). Oral killed whole cell vaccines cause no untoward reactions but are not protective (2). Thus, live oral Ty21a vaccine, which is efficacious without causing adverse reactions, represents a major breakthrough in immunization against typhoid fever. A preliminary field trial carried out by Wahdan et al (16,17) showed that under some conditions three doses of Ty21a vaccine can provide outstanding (96%) efficacy for at least three years. However, the formulation utilized in the Alexandria field trial was a one time affair that was not amenable to large scale production nor was it practical for mass vaccinations. Furthermore, the annual incidence of typhoid fever in the control group in Alexandria was well below rates encountered in most typhoid-endemic areas and it was critical to assess the efficacy of Ty21a in a site with a more potent force of infection.

A series of three large-scale field trials carried out in Santiago, Chile has brought Ty21a vaccine to the point where firm recommendations can be made for its use in control of endemic typhoid fever. From these trials it was learned that an enteric-coated formulation which is highly practical for mass immunizations of schoolchildren is significantly superior to a formulation consisting of gelatin capsules that contain NaHCO_3 and vaccine. Poor

protection with the gelatin capsule formulation has also been reported in a retrospective study in Swiss travelers by Hirschel and Vuthrich.¹⁶ It has also been learned that doses of vaccine need not be widely spaced and that two and three doses, but not one dose, of enteric-coated vaccine can confer circa 60% protection. The field trial initiated in Area Sur and Area Central in 1984 will determine whether there is a significant difference in the protection conferred by two, three or four doses of vaccine. Continued surveillance in the field trial areas is being maintained to answer the question of duration of efficacy.

A sobering observation made in the first field trial of Ty21a in Chile is that under certain epidemiologic conditions the protective effect of two doses of Ty21a vaccine can be overcome. Studies by Hornick et al (9) in volunteers showed that the efficacy of two parenteral killed whole cell typhoid vaccines was related to the challenge inoculum ingested. When a dose of pathogenic S. typhi was ingested that caused typhoid fever in 25% of control volunteers, vaccine efficacy was circa 79%. However, when a 95% infectious dose was ingested, vaccine efficacy dropped to 25% in the volunteers. It is conceivable that the lack of vaccine efficacy encountered during the three month periods of April to June to March 1985 was due to ingestion of unusually high inocula.

The immunologic mechanisms by which Ty21a protects are just beginning to be intensively studied. It is believed that cell-mediated immunity is most critical. Currently, studies are in progress measuring the cell-mediated immune response to Ty21a vaccine to allow documentation of a vaccine "take". This is an important area of investigation because subtle changes in formulation, dosage, immunization schedule, etc., can effect immunogenicity and it is not feasible to carry out a large-scale field trial each time such a

modification is made. So a reliable and predictable assay is being sought to correlate immune response with protection.

In a period of 12 years from the first administration of Ty21a to volunteers in 1973, the vaccine has reached the point of being ready for use in public health. The vaccine strain has been shown to be safe, protective, and amenable to mass vaccination when formulated in enteric-coated capsules. Certain other attenuated S. typhi strains, such as auxotrophic mutants (14), have been prepared by more precise genetic manipulations leading to defined genetic deletions. Whatever attenuated strains come to be the most frequently used vaccine of the future, it is Ty21a that has served as the pathfinder and prototype in demonstrating the attributes and advantages of live oral typhoid vaccines under field conditions.

Acknowledgements

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Table 1

Field trial of efficacy of three doses of a liquid formulation of Ty21a vaccine given with NaHCO₃ to six and seven year old schoolchildren in Alexandria, Egypt. Results of three years of surveillance.^a

<u>Year of observation</u>	<u>Confirmed cases of typhoid fever</u>	<u>Annual incidence per 10⁵</u>	<u>Vaccine efficacy (%)</u>
1978-1979 ^b vaccinees	0	0	100
placebo ^c	7	44	
1979-1980 vaccinees	0	0	100
placebo	8	50	
1980-1981 vaccinees	1	6	86
placebo	7	44	

^aM. Mahdian et al. J Infect Dis 145:292, 1982.

^bn=16,486

^cn=15,902

Table 2

Randomized, placebo-controlled, double blind clinical trials of three doses of Ty21a in enteric-coated or in milk with NaHCO₃ or placebo to assess reactogenicity and immunogenicity

Adverse Reactions ^a	Adults		Children		
	Vaccines (384)	Placebo (367)	Enteric coated vaccine (172)	Vaccine in milk with NaHCO ₃ (165)	Placebo (172)
Abdominal pain	4.9% ^b	2.5	6.4	11.5	17.4
Diarrhea	1.8	1.1	1.2	6.0	9.9
Vomiting	0.5	0.3	2.3	7.3	11.0
Fever	0.3	0.5	0.6	1.2	0.6
Headache	4.7	3.8	ND ^c	ND	ND
Skin rash	0.5	0.5	ND ^c	ND	ND
O antibody seroconversions	ND	ND	53	57	0

^aNo adverse reactions occurred significantly more frequently in vaccinees than placebo controls.

^bPercent positive of total individuals in the group.

^cNot determined.

Table 3

Field trial of efficacy of one or two doses of Ty21a oral typhoid vaccine in enteric-coated capsules. 33 months of surveillance,

	Placebo (31,762)	Two Dose Vaccinees (27,485)			One Dose Vaccinees (32,707)			
	Cases ^a	Rate/10 ⁵	Cases	Rate/10 ⁵	Vaccine Efficacy (%)	Cases	Rate/10 ⁵	Vaccine Efficacy (%)
Year 1 7/82-6/83	67	210.9	29	105.5	50	58	177.3	
Year 2 7/83-6/84	45	141.7	11	40.0	72	27	82.6	
Year 3 ^b 7/84-3/85	12	37.8	9	32.7	13	16	48.9	
Total 33 months	124	390.4	49	178.3	54	101	308.8	

^a Cases are confirmed by blood or bone marrow culture.

^b Only 9 months of surveillance.

Table 4

EFFICACY OF ONE OR TWO DOSES OF TY21A ORAL TYPHOID VACCINE IN ENTERIC-COATED CAPSULES IN RELATION TO AGE. 33 MONTHS OF SURVEILLANCE, AREA NORTE, SANTIAGO, CHILE FIELD TRIAL, JULY 1982 TO MARCH 1985

Age Group (N) ^a	Two Dose Vaccinees				One Dose Vaccinees			
	Placebo		Vaccine		Placebo		Vaccine	
	Cases ^b	Rate/10 ⁵	Cases ^b	Rate/10 ⁵	Cases ^b	Rate/10 ⁵	Cases ^b	Rate/10 ⁵
5-9 yrs. (16,613)	42	737.1 ^a	19	383.5 ^b	48	587.2	20	328.5
10-14 (35,604)	60	888.6 ^c	24	222.3 ^d	55	447.0	9	35.5
15-19 (38,758)	24	179.4 ^e	8	69.3 ^f	61	115.7		

^aExcludes 679 children for whom exact age was not recorded.

^bOnly blood or bone marrow culture confirmed cases included.

a vs b, p<0.025

c vs d, p=0.018

d vs e, p<0.002

e vs f, p=0.025

b vs d, p=0.018

d vs f, p=0.025

b vs f, p<0.0001

Table 5
Field trial of Ty21a vaccine in enteric coated capsules
formulation, Area Norte, Santiago, Chile.
Efficacy by three month intervals of surveillance.

	Placebo (31,762)		Two dose vaccinees (27,485)		Vaccine efficacy (%)
	Cases	Rate/10 ⁵	Cases	Rate/10 ⁵	
1982					
July-Sept	1	3.1	0	0	100
Oct-Dec	13	40.9	4	14.6	64
1983					
Jan-Mar	36	113.3	10	36.4	68
Apr-June	17	53.5	15	54.6	0
July-Sept	4	12.6	1	3.6	71
Oct-Dec	14	44.1	1	3.6	92
1984					
Jan-Mar	19	59.8	6	21.8	64
Apr-June	8	25.2	3	10.9	57
Jul-Sept	1	3.1	0	0	100
Oct-Dec	5	15.7	1	3.6	77
1985					
Jan-March	6	18.9	8	29.1	0

EFFICACY OF TY21A VACCINE AGAINST SALMONELLA PARATYPHI B INFECTION. AREA NORTE, SANTIAGO, CHILE FIELD TRIAL, JULY 1932 TO APRIL 1985

^aIncludes only blood or bone marrow culture confirmed cases.

Table 7

Efficacy of Ty21a oral typhoid vaccine in Area Occidente field trial after 18 months of surveillance (September 1983 to March 1985). Comparison of two different formulations and immunization schedules

Group	No. children ^a	No. ^b cases	Rate per 10 ⁵	Vaccine efficacy
<u>Three doses, two day interval between doses</u>				
Enteric-coated formulation	22,170	15	67.7 ^c	62%
Gelatin/NaHCO ₃ formulation	22,379	33	147.5 ^d	18%
<u>Three doses, 21 day interval between doses</u>				
Enteric-coat formulation	21,598	16	74.1 ^e	59%
Genetic/NaHCO ₃ formulation	21,541	27	125.3 ^f	30%
Placebo	27,793	50	179.9 ^g	
Non-vaccinated	14,962	27	108.5	

^a25,646 additional children received only one or two doses of vaccine.

^bBacteriologically confirmed by blood or bone marrow culture.

^c vs ^g, $p < 0.003$

^c vs ^d, $p = 0.015$

^e vs ^g, $p < 0.007$

^{c+e} vs ^{d+f}, $p < 0.004$

Table 8

Comparison of the efficacy of two, three or four doses

of Ty21a oral typhoid vaccine in enteric-coated formulation.

Nine months of surveillance in Area Sur/Central, 11/84 to 7/85.

<u>Vaccine Group</u>	<u>N</u>	<u>No. culture-confirmed cases of typhoid</u>	<u>Incidence per 10⁵</u>	<u>Vaccine efficacy</u>
Not vaccinated	31,681*	18	56.8	-
Two doses	94,387	46	48.7 ^a	14%
Three doses	95,543	38	39.8 ^b	30%
Four doses	58,614	11	18.8 ^c	67%

a vs c $p < 0.005$

b vs c $p < 0.04$

*This was not a randomized group.

APPENDIX B

EPIDEMIOLOGIC INVESTIGATION OF FOODHANDLERS
IN SCHOOLS IN A TYPHOID ENDEMIC AREA

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ABSTRACT

In Santiago, Chile, an area hyperendemic for typhoid fever, the peak incidence occurs in schoolchildren. Although typhoid fever is most common during summer when children are on school holiday, children are nevertheless in school during a portion of the typhoid season. Previous epidemiologic studies (Black R et al., Bulletin of the World Health Organization, in press) have identified eating lunch in school and sharing lunch at school with classmates as significant risk factors for development of typhoid fever. Accordingly, we undertook epidemiologic studies in schools of foodhandlers. These included: 1) a search for chronic Salmonella typhi carriers (by two stool cultures and Vi serology); 2) assessment of the knowledge of foodhandlers regarding modes of transmission of enteric infections 3) quantitation of the personal hygiene of foodhandlers using the presence of coliforms or fingernail cultures as a marker.

Of 137 foodhandlers who had stool cultures, only one (0.7%) S. typhi and one S. paratyphi B carriers were found. Fifty-eight % of foodhandlers were unaware of how typhoid fever is spread. Coliforms were cultured from 79 of 103 school foodhandlers (77%) but from only 15 of 92 (16%) controls with other occupation ($p < 0.001$), suggesting a poor level of personal hygiene. The general condition of school kitchens required improvements: 25% had evidence of mice, 33% had flies, 12% of bathrooms had no soap or towels.

In summary, although levels of hygiene were faulty in kitchens and among foodhandlers, chronic S. typhi carriers were rare, school foodhandlers cannot be incriminated as an important source of spread of S. typhi.

In Santiago, Chile, typhoid fever has surprisingly remained endemic over the past 15 years (1-4), despite marked improvements in sanitary conditions wherein 94% of households now have chlorinated, bacteriologically monitored drinking water and 75% are connected to the municipal sewerage system (5,6). The age group affected is 6-19 years, i.e. the schoolchild, but the peak incidence of typhoid occurs in January and February when children are on summer holiday (1-4).

In recent years in Chile it has been taught that the main mechanism of transmission of typhoid infection involves a chronic carrier directly contaminating food vehicles (2,7). However, several recent epidemiologic studies that have attempted to confirm this mode of transmission have, with rare exceptions, failed to detect chronic Salmonella typhi carriers in the households of index case schoolchildren (6,8). The observations made in these previous studies (6,8) suggested that in school age children the consumption of contaminated vehicles must be occurring mainly outside the household. Although most typhoid fever transmission occurs while children are on holiday from school, among the risk factors for typhoid fever that were implicated in a case/control study (8) in Santiago were: 1.) sharing foods at school with classmates; 2.) eating lunch at school.

Accordingly we undertook studies of the sources of food consumed by children in Santiago schools and of the hygiene associated with food preparation and distribution. The specific objectives of the study included:

- 1) To determine the school-related sources of food for schoolchildren in a representative sample of Santiago schools.
- 2) To identify foodhandlers within the schools as well as those on the street in front of the school and to determine their knowledge of the causes and modes of transmission of enteric infection.

4) To measure the prevalence of chronic carriers of S. typhi and S. paratyphi among school foodhandlers and relate this to the school-specific incidence of typhoid fever.

MATERIALS AND METHODS

Since 1964 there has existed a school feeding program in Chile which provides free food to 6-14 year old schoolchildren of the lower socioeconomic level. From the total of 703 schools in Santiago that receive such assistance, we randomly selected 77 (11%) of the schools for study; the sample was stratified by counties.

Health Inspection of Kitchens

Two trained public health nurses visited the selected schools and performed a systematic health inspection of the kitchens.

Identification of Foodhandlers

The nurses registered and interviewed all persons who were involved in food preparation for the schoolchildren, whether in the school kitchen, or food kiosks, (these are small booths found in many schools where candy, cookies, soft drinks, and occasionally, sandwiches are sold). Street vendors found within 100 meters of the entrances of the schools were also registered. A questionnaire was administered to all the foodhandlers to obtain information regarding a past history of typhoid fever and to assess their basic knowledge of enteric diseases and their transmission.

Bacteriologic and Serologic Studies in Foodhandlers

For those foodhandlers who were directly involved in the preparation of food (all kitchen workers and those food kiosk workers who prepared sandwiches) the following bacteriologic studies were performed:

a) Stool cultures to identify Salmonella:

Two stool samples were taken every other day and transported in Cary Blair transport medium to the Institute of Public Health where they were

cultured for Salmonella and Shigella by standard methods (14).

b) Fingernail cultures for enteric bacteria as a measure of personal hygiene:

From a random sample of foodhandlers, cultures were obtained by direct impression of the fingers onto a MacConkey's agar plate (a differential medium for identifying enteric bacteria).

In order to better help determine the significance of the observations, we also cultured the fingernails of a control group composed of persons of comparable age, sex, and economic level but with different employment.

c) Serology to detect chronic typhoid carriers:

One blood sample was obtained to determine the levels of serum antibodies to the Vi antigen of Salmonella typhi, a sensitive and specific screening test to detect chronic typhoid carriers. Vi antibodies were measured by passive hemagglutination technique using highly purified Vi antigen (kindly provided by Dr. John Robbins, Bethesda, Md.) as previously described (15,16).

RESULTS

Health Inspection of Kitchens

In general, the physical environment and facilities in the school kitchens were adequate and a high standard of cleanliness was evident. However, deficiencies or substandard conditions were noted in some schools: in 25% of the kitchens the presence of mice was detected and in one-third, house flies; only one kitchen had an adequate garbage disposal system; in 12% of the bathrooms used by the kitchen staff there was no soap or towels.

Identification of Foodhandlers

In the 77 schools visited, 167 individuals were identified who prepare or dispense food; 121 (73%) worked in the school kitchen and 35 (21%) in snack kiosks. In addition 11 street vendors were identified, all near the

entrances to the schools. The ages of the food handlers, 98% of whom were women, ranged from 17 to 69 years (Table 1); 6% gave a past history of typhoid fever.

Among the food handlers, 40% had previously participated in a food hygiene course. When asked about enteric infections, 95% of the foodhandlers answered correctly that typhoid is contagious but only 25% knew the modes of transmission and only 7% correctly described methods to prevent the spread of typhoid fever.

Personal Hygiene of Foodhandlers

With respect to personal hygiene, it was observed that only 50% of foodhandlers had short, clean fingernails. Of the 137 food handlers involved in food preparation (121 kitchen and 16 food kiosk workers), it was possible to culture the fingernails of a random sample of 99. Of these 99 (84 kitchen and 15 kiosk workers), 79 (80%) grew enteric bacteria. In contrast, enteric bacteria were cultured from the fingernails of only 15 of 92 office worker controls (16%) ($p < 0.001$). Since salad vegetables cultivated in the metropolitan Santiago area are known to be heavily contaminated with enteric bacteria as a consequence of irrigation with raw sewage (17), such vegetables could be responsible for contaminating the hands of foodhandlers. Therefore the frequency of use of fresh salad vegetables in school lunch program was investigated. It was found that uncooked salad vegetables (such as lettuce or celery) are never included as part of the school lunch provided by the feeding program in Santiago schools. Thus foodhandlers did not manipulate raw salad vegetables with their hands in school kitchens.

Detection of Typhoid Carriers

All 137 foodhandlers who prepared food had stool cultures. Salmonella were detected in only two individuals (1.5%). Salmonella typhi was recovered from the stool culture of a 38 year old women who had been hospitalized for

typhoid fever in 1981, while from another healthy 43 year old woman, who gave no past history of enteric fever, Salmonella paratyphi B was isolated. From a third healthy 59 year old woman Shigella sonnei was cultured.

The serologic screening test for chronic typhoid carriers was performed on sera of 136 of the 137 foodhandlers who prepared food (16).

Two women had elevated reciprocal titers of 80. One corresponded to the Salmonella typhi chronic carrier who was also detected by stool cultures. The other elevated Vi titer belonged to a 30 year old woman who had four negative stool cultures and refused further bacteriologic examination.

School-Specific Incidence of Typhoid Fever

In order to assess the epidemiologic importance of the detection of chronic carriers of S. typhi and S. paratyphi among foodhandlers, the incidence of bacteriologic proven cases of typhoid fever as compared between the school with the chronic S. typhi carrier and 7 other schools, of the same county, where no carriers were found among the foodhandlers. For this analysis we selected the period September 1983 to January 1984 when all these schools were under the same typhoid fever surveillance system. In the seven schools without known chronic carriers, 10 cases of typhoid were confirmed among the children at risk, a rate of 2.6 per 1000. This rate closely resembles that in the school with the detected chronic typhoid carrier foodhandler (2.9 per 1000), (Table 2).

DISCUSSION

The modes and vehicles of transmission of S. typhi in endemic areas are multiple and complex, making their identification notoriously more difficult than in the investigation of outbreaks of typhoid fever in non-endemic areas. Since typhoid fever has its highest incidence in school age children in Santiago, Chile, it behooved us to investigate food hygiene and foodhandlers, even though the highest incidence of typhoid fever occurs while children are on school

holiday. This was also indicated since folklore in Santiago has traditionally incriminated street vendors who sell snacks in front of schools as being important in the transmission of typhoid fever.

The systematic study of school kitchen hygiene and of foodhandlers has provided some notable observations. For the first time there has been provided a clear elucidation of the number and type of food-handlers who impinge upon children in typical Santiago schools. Surprisingly, street vendors were found to handle mostly packaged foods, (candy, etc.) that must be considered at low risk of contamination. The general level of hygiene in school kitchens was high. However, foodhandlers were found to have poor knowledge of the modes of transmission of typhoid fever and means to prevent it. Furthermore, a large proportion of foodhandlers had bacteriologic evidence of fecal contamination of their hands as determined by fingernail cultures yielding coliform bacteria.

The Vi serology successfully detected the one chronic S. typhi carrier who was also detected by multiple stool cultures of each food preparer. Multiple stool cultures are impractical, expensive and require considerable technician time to process. The Vi serology, in contrast, is inexpensive, sensitive, specific and amenable to screening large numbers of specimens. The result of this survey suggest that the Vi serology with purified antigen should be utilized as an economical and efficient screening test to identify chronic carriers of S. typhi, reserving bacteriologic cultures for those persons with elevated Vi titers.

Although the survey showed poor personal hygiene by many school food preparers, poor knowledge of the modes of transmission of S. typhi and of its prevention, and the existence of one chronic S. typhi carrier among the foodhandlers, there was no epidemiologic evidence that this carrier was responsible for any cases in the school. During the same time period and under the same surveillance system,

there was a close similarity in the incidence of typhoid fever in the school with the carrier ($2.9/10^3$ schoolchildren) and in the seven schools without carriers ($2.6/10^3$) (Table 2). While this appears to be true, the observations made during this investigation nevertheless warrant consideration for instituting more vigorous daily hand-washing by school foodhandlers as well as a health education program to teach them the important diseases that can be transmitted by food and simple measures to prevent food-borne transmission.

In summary, the systematic investigation of food hygiene and food handlers in Santiago schools has failed to either find a high prevalence of typhoid carriers or to incriminate foodhandlers as an important source of transmission of S. typhi. This is consistent with the observation that the majority of cases of typhoid fever in fact occurs while children are on summer holiday and thus involves modes of transmission unrelated to schools. Further epidemiologic investigations will concentrate on the search for risk factors and vehicles of transmission operative during the school holiday season. If these factors can be clearly identified, it may be possible to institute specific interventions and preventive measures.

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Table 1. Age distribution of foodhandlers in schools
in Santiago, Chile, 1983

<u>Age Group</u>	<u>Number of Foodhandlers (%)</u>	<u>No (%) Positive for Bacterial Pathogens</u>
15-19	4 (2.4)	0
20-24	8 (4.8)	0
25-34	31 (18.6)	0
35-44	45 (26.9)	2* (4.4)
45-54	43 (27.7)	0
55-64	43 (19.2)	1+ (3.1)
≥65	3 (7.8)	0
Unknown	1 (0.6)	0
	167 (100.0)	3 (1.8)

* 1 S. typhi and 1 S. paratyphi

+ Shigella sonnei

Table 2. The incidence of typhoid fever among schoolchildren and its relation to chronic S. typhi carriers in the school kitchens.

	<u>Schools without chronic carriers</u>	<u>Schools with chronic S. typhi carriers</u>
No. of schoolchildren	3348	334
Confirmed cases of typhoid fever	9	1
Attack rate/10 ³	2.6	2.9

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